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TOPICAL REVIEW

Measuring cerebrovascular reactivity: what stimulus to use?

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Abstract Cerebrovascular reactivity is the change in cerebral blood flow in response to a vasodilatory or vasoconstrictive stimulus. Measuring variations of cerebrovascular reactivity between different regions of the brain has the potential to not only advance understanding of how the cerebral vasculature controls the distribution of blood flow but also to detect cerebrovascular pathophysiology. While there are standardized and repeatable methods for estimating the changes in cerebral blood flow in response to a vasoactive stimulus, the same cannot be said for the stimulus itself. Indeed, the wide variety of vasoactive challenges currently employed in these studies impedes comparisons between them. This review therefore critically examines the vasoactive stimuli in current use for their ability to provide a standard repeatable challenge and for the practicality of their implementation. Such challenges include induced reductions in systemic blood pressure, and the administration of vasoactive substances such as acetazolamide and carbon dioxide. We conclude that many of the stimuli in current use do not provide a standard stimulus comparable between individuals and in the same individual over time. We suggest that carbon dioxide is the most suitable vasoactive stimulus. We describe recently developed computer-controlled MRI compatible gas delivery systems which are capable of administering reliable and repeatable vasoactive CO₂ stimuli.

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Abbreviations ACZ, acetazolamide; ASL, arterial spin labeling; BOLD, blood oxygen level-dependent; CBF, cerebral blood flow; CVR, cerebrovascular reactivity; DEF, dynamic end-tidal forcing; MRI, magnetic resonance imaging; SGD, sequential gas delivery (circuit); TCD, trans-cranial Doppler.

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Introduction

Cerebral blood flow (CBF) is carefully regulated to provide nutrients and oxygen to the various regions of the brain. Metabolic coupling mechanisms ensure that blood flow is increased in active regions (Iadecola & Nedergaard, 2007; Attwell *et al.* 2011; Paulson *et al.* 2011), and autoregulation ensures that blood flow is maintained in the face of changes in perfusion pressure (Lucas *et al.* 2010). The cerebral vasculature also responds to carbon dioxide and hypoxia (Kety & Schmidt, 1948; Mardimae *et al.* 2012). The action of these various factors serves to control the distribution of cerebral blood flow. One of the ways investigators have probed the regulation of cerebral blood flow and its distribution is to challenge the system with a vasoactive stimulus and observe the response. Such experiments measure the cerebrovascular reactivity (CVR), the change in CBF per change in vasoactive stimulus.

For example, recent studies using trans-cranial Doppler (TCD) measurements of CBF and carbon dioxide (CO_2) as a vasoactive stimulus have found differences in CVR between the major cerebral arteries (Sato *et al.* 2012; Willie *et al.* 2012). And, as Fig. 1 shows, maps of CVR obtained from blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI), which is used as a surrogate for cerebral blood flow, can detect inhomogeneities in the distribution of cerebral blood flow. The BOLD signal is degraded in proportion to the concentration of deoxyhaemoglobin in a voxel. It represents the net balance between O_2 delivery and O_2 consumption. At physiological levels of tissue blood flows, the BOLD signal and

blood flow approximate a linear relationship (assuming O_2 consumption is constant). Measuring variations of cerebrovascular reactivity between different regions of the brain has the potential to not only advance understanding of how the cerebral vasculature controls blood flow and its distribution but also to detect cerebrovascular pathophysiology (e.g. Mandell *et al.* 2011).

However, currently published CVR studies use different combinations of vasoactive stimuli and measures of CBF, generating results that are difficult to compare. Although the methods of measuring CBF differ in their sensitivity, specificity, and spatial and temporal resolution, each does, nevertheless, generate highly repeatable and specific measures (see Wintermark *et al.* 2005 for review). The same cannot be said of the vasoactive stimulus. The many vasoactive stimuli that have been used to increase CBF generate results that are difficult to compare between studies. Even within a single method, the vasodilatory effect is seldom uniform, repeatable, or even quantifiable. It is this inability to standardize the vasoactive stimulus, or alternatively, precisely and accurately measure the stimulus at the active site, that has most impeded comparison of CVR between various studies, and between individuals in the same study.

We therefore set out to survey vasoactive stimuli used to generate CVR measures. We conclude that CO_2 is the most appropriate stimulus in terms of its practicality of use and ability to be standardized. Since CBF responds to arterial CO_2 tension, we review methods of controlling the arterial partial pressure of CO_2 ($P_{\text{a,CO}_2}$), specifically commenting on their anticipated advantages and limitations used for making CVR measurements. We then present a detailed description of an automated prospective method of targeting $P_{\text{a,CO}_2}$ developed in our laboratory. We note that the various methodologies for controlling $P_{\text{a,CO}_2}$ are complex and require some knowledge of respiratory physiology in order to appreciate the subtleties involved. Accordingly, we provide an Appendix containing a brief glossary and summary of the basic principles of respiratory physiology pertinent to the control of CO_2 as a vasoactive stimulus.

Vasodilatory challenges

The main types of vasoactive stimuli. To measure CVR, three types of vasodilatory challenges have been utilized: (1) the application of a transient reduction in mean arterial blood pressure (MacKenzie *et al.* 1979), (2) the injection of an exogenous chemical (e.g. acetazolamide; Vorstrup *et al.* 1986) and (3) an increase in $P_{\text{a,CO}_2}$ (e.g. Poulin *et al.* 1996). The ease of use of each method as a standardized stimulus is assessed in the following sections.

Systemic blood pressure as a stimulus. At first glance, a reduction in systemic blood pressure (Panerai, 1998)

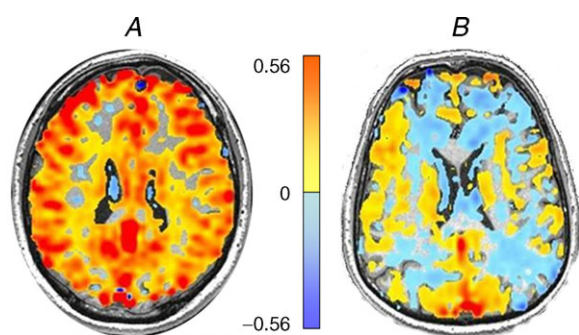


Figure 1. Example CVR maps

CVR is the ratio of the change in the BOLD-MRI signal in response to a change in CO_2 stimulus, and is colour coded voxel by voxel according to the scale provided in the colour bar, and superimposed on the corresponding voxel of the anatomical scan. A, CVR map measured in a healthy volunteer. B, CVR map measured in a patient with moyo moyo disease. Although the hypercapnic stimulus was the same in each subject, the distribution of the changes in blood flow in the patient with moyo moyo disease revealed multiple areas of reduced cerebrovascular reactivity in response to the global vasodilatory stimulus. These regions of reduced CVR are the result of vascular steal, where stimulus-induced reductions in flow resistance result in flow diversion from regions of low to high vasodilatory reactivity.

appears to be the ideal vasodilatory challenge for testing the basic autoregulatory response of the cerebral vasculature. However, the induction of a safe, titratable and repeatable hypotensive stimulus is challenging. Inducing hypotension by administering vasodilators makes it difficult to separate the vascular effects resulting from autoregulation and those resulting from the direct effect of vasodilators (Artru & Colley, 1984). Abrupt thigh cuff release (single step change in blood pressure; Aaslid *et al.* 1989) and lower body negative pressure (cyclic changes in blood pressure; Tan, 2012) are designed to examine dynamic cerebral autoregulation. Tan *et al.* (2012), for example, examine the loss of coherence between arterial pressure changes and TCD signal changes as an index of autoregulation. However, these methods reduce blood pressure through a reduction in venous return and effectively central blood volume. The extent of the ensuing hypotension in healthy subjects is variable (Mahony *et al.* 2000), and is expected to be more variable if performed in patients over a range of ages, with multiple underlying co-morbidities and taking an array of medication. Hypotensive provocation would also be a concern for patients at risk of hypotension-induced ischaemia such as those with already haemodynamically precarious regional brain blood flow (Symon, 1969; Mandell *et al.* 2008).

Hypotension vs. chemical stressors. These considerations regarding hypotension have resulted in a preference for the administration of chemical cerebral vasodilators to test vasodilatory reserve in clinical studies. To do so, investigators had to address the concern that the mechanism of vasodilatation due to autoregulation may not be the same as for a chemical stimulus such as acetazolamide (ACZ) or CO₂ (Lundar *et al.* 1985). The conclusion of studies performed over the last half-century is that regardless of the underlying mechanisms, cerebrovasodilators and pressure autoregulation draw on the same vasodilatory reserve (Symon, 1968; Nishimura *et al.* 1999). Reductions in perfusion pressure, resulting in autoregulatory vasodilatation, progressively dampen both the vasoconstrictor (Artru & Colley, 1984) and vasodilatory responses to CO₂ in dogs (Harper, 1965) and humans (Vorstrup *et al.* 1986; Ringelstein *et al.* 1992; Nishimura *et al.* 1999). Overall, these studies indicate that chemical stimuli, and in particular hypercapnia, are suitable for interrogating the brain vasculature for regions with limited vasodilatory capacity.

Exogenous chemical acetazolamide as a stimulus. In 1986, Vorstrup *et al.* (1986) introduced the use of intravenous administration of 1 g of the carbonic anhydrase inhibitor ACZ as a maximal vasodilatory stimulus for measuring CVR. Indeed, ACZ continues to be the most

widely applied stimulus for cerebral vasodilatation in a clinical setting and is used with TCD (Ringelstein *et al.* 1992; Gur *et al.* 1996), ¹³³Xe enhanced-CT (Yonas *et al.* 1993), single positron emission computed tomography (SPECT), (Hasegawa & Yamaguchi, 1993; Kuroda *et al.* 2001) and BOLD MRI (Asghar *et al.* 2011). ACZ administration produces an extracellular and intracellular acidosis resulting in the relaxation of vascular smooth muscle. The advantages claimed for ACZ as a cerebral vasodilator are the simplicity of a single dose injection (Okazawa *et al.* 2001), its reproducibility independent of subject cooperation, and its high safety profile.

However, a standardized dose of ACZ does not generate the reproducible stimulus required for standardizing CVR measurement. First, there is considerable individual variability in the response to ACZ; both the serum ACZ concentrations for a given dose, and the cerebrovascular responses to a given serum level, vary between subjects. These unpredictable and uncontrollable influences prevent a reproducible stimulus–response relationship. It has been suggested that the best way to standardize the use of ACZ for CVR is to give a large enough dose to obtain a maximal CBF response. However, this approach also poses difficulties as there are large inter-subject variations in ACZ's time to peak effect, duration of action, and elimination rate. While a single 1 g dose of ACZ, is assumed to provide a supramaximal stimulus (Ringelstein *et al.* 1992; Okazawa *et al.* 2001), this single dose does not reliably produce a maximum CBF if it is less than 15 mg kg⁻¹ (Dahl *et al.* 1995; Grossmann & Koeberle, 2000). Even with an effective supramaximal ACZ dose, CBF continues to respond to ventilation-induced changes in P_{CO₂} and to changes in blood pressure (Vorstrup *et al.* 1986); both are confounding factors affecting CBF. Second, doses in the 15 mg kg⁻¹ range are associated with a high incidence of side-effects, including dizziness, nausea, vomiting, headache, malaise and neurological symptoms related to ischaemia (Dahl *et al.* 1995; Grossmann & Koeberle, 2000). Even at lower doses there are symptomatic complaints following ACZ in 40–60% of subjects (Ringelstein *et al.* 1992; Saito *et al.* 2011). A major disadvantage of ACZ is that in case of adverse events or subject discomfort the effects are not easily terminated. Altogether, these issues limit the desirability of ACZ as a vasoactive stimulus. An alternative stimulus is needed. On the basis that CVR measures using CO₂ are similar to those using ACZ, (Markus & Harrison, 1992; Ringelstein *et al.* 1992; Gooskens *et al.* 2003), it is reasonable to consider CO₂ as a suitable candidate for the vasodilatory stimulus.

Carbon dioxide as a stimulus. CO₂ as a vasodilatory stimulus has many advantages. Increases in P_{a,CO₂} raise CBF by about 3% (Fortune *et al.* 1992) or more under

hypoxic conditions (Poulin *et al.* 2002), and in contrast to intravenously injected ACZ, the administration of CO₂ is non-invasive and easily terminated. CBF closely follows changes in P_{a,CO_2} for TCD measurements of the middle cerebral artery flow velocity (Poulin *et al.* 1996), and even faster for BOLD MRI measures as we show later. The physiological mechanisms of vasodilatation with CO₂ have been reviewed (Brian, 1998). Moderate hypercapnia with end-tidal P_{CO_2} (P_{ET,CO_2}) controlled at tensions between 40 and 50 mmHg is well tolerated by conscious humans (Steinback *et al.* 2009; Spano *et al.* 2012). Furthermore, CO₂ administration is safe even in critically ill patients, and even when applied for prolonged periods (e.g. weeks; Feihl & Perret, 1994; O'croinin *et al.* 2005). A further indication of the safety of hypercapnia comes from case reports of accidental extreme hypercapnia; patients have recovered from P_{a,CO_2} values exceeding 200 mmHg without adverse neurological sequelae after nothing more than restoration of normal ventilation (Potkin & Swenson, 1992; Ayas *et al.* 1998).

Despite these advantages, few clinical studies have considered using CO₂ as the vasoactive stimulus and its use is mostly in laboratory studies. One of the reasons cited against using CO₂ is that changing P_{a,CO_2} requires some type of ventilatory manoeuvre such as breath-holding or voluntary hyperventilation, and therefore some cooperation from the subject, as opposed to ACZ, which can simply be injected intravenously. Furthermore, the perception is that the extent of the stimulus cannot be as easily regulated as drug dosing. Indeed, this latter difficulty in controlling P_{a,CO_2} has been an incentive to develop new technology for targeting and maintaining P_{a,CO_2} tension.

Whether to use hypocapnia or hypercapnia is the first consideration. Hypocapnia has a vasoconstrictive effect on the brain vasculature, thereby reducing cerebral blood flow (Raichle & Plum, 1972) and has been used for CVR mapping (Bright *et al.* 2009). However, Ringelstein *et al.* (1988, 1992) induced hypocapnia for clinical CVR testing by hyperventilation in patients with an occlusion of the internal carotid artery and found that, in comparison with a hypercapnic stimulus and acetazolamide, hypocapnia led to a relatively blunted response of CVR. Furthermore, a hypocapnic manoeuvre for CVR testing in subjects with traumatic brain injury led to an even more heterogeneous response (Steiner *et al.* 2005). It also seems counterintuitive to reduce CBF by hyperventilation for identifying patients at risk for cerebral ischaemia. Therefore, a hypercapnic vasodilatory stimulus is more often applied for clinical CVR examinations. Before describing these new approaches for controlling P_{a,CO_2} , we review specific methods employed in recently published studies to produce hypercapnia. Please refer to the Appendix for an explanation of the terms and respiratory physiology involved.

Hypercapnia

Breath-holding. One of the earliest methods used to induce hypercapnia was breath-holding to prevent CO₂ elimination, which results in a progressive increase in P_{a,CO_2} with time. The use of breath-holding for CVR measurement was first demonstrated by Ratnatunga & Adiseshiah (1990), used clinically with TCD measurements of blood velocity to assess the risk of stroke in symptomatic patients with carotid artery disease (Markus & Harrison, 1992; Kastrup *et al.* 1999, 2001; Silvestrini *et al.* 1999, 2000; Vernieri *et al.* 1999). In these studies, breath-holding time was taken as an indicator of the strength of the vasoactive stimulus and used to normalize the change in CBF signal for stimulus strength, and establish a 'breath hold index' (Kastrup *et al.* 1999; Silvestrini *et al.* 1999, 2000; Vernieri *et al.* 1999).

The problem with this approach is the tenuous relationship between breath-holding time and the actual P_{a,CO_2} stimulus controlling CBF: breath-holding time is only one of several factors determining the actual change in P_{a,CO_2} . Other factors include the metabolic rate of the subject, the size of the lungs (which vary with age, sex, height, abdominal girth, body position, extent of chest compression with fat, presence of obstructive and other lung diseases etc.), recent ventilation history (e.g. if preceded by hyperventilation, the P_{a,CO_2} may change little if at all), and whether the subject inspired or expired immediately before breath-holding. Even if these factors are somehow controlled, P_{a,CO_2} is still not a linear function of breath-holding time as has been assumed (Sasse *et al.* 1996; Stock *et al.* 2006). Finally, the duration of breath-holding is highly variable between subjects, varying by more than 4 times (see Table I of Markus & Harrison, 1992), even within the same subject (Totaro *et al.* 1999). All of these factors contribute to a large variability and non-linear relation between breath-holding times and the resulting P_{a,CO_2} stimulus; an issue that cannot be addressed if CVR measures are to be comparable. While breath-holding techniques can be refined (Scouten & Schwarzbauer, 2008; Murphy *et al.* 2011; Bright & Murphy, 2013), by including end-tidal measures for example, they have not been shown to provide CVR maps that reliably identify cerebrovascular pathology because the threshold stimulus for all pathological conditions is still unknown. These drawbacks make breath-holding unsuitable as a method of producing a standardized hypercapnic stimulus for CVR measurements.

Inspired CO₂. A second commonly used method of increasing P_{a,CO_2} is the administration of a fixed inspired fractional concentration of CO₂. Breathing 2, 5 or 7% CO₂ via a tight fitting non-rebreathing face mask is often considered to produce a standard hypercapnic stimulus (Bishop *et al.* 1987; Ringelstein *et al.* 1988, 1992; Hoge *et al.* 1999; Vernieri *et al.* 2004). However, despite

widespread confidence in this approach, a fixed inspired fractional concentration of CO_2 does not produce a fixed related $P_{\text{a,CO}_2}$, but one that varies both between subjects and within the same subject over time (Hoskins, 1990; Prisman *et al.* 2008). Indeed, the resulting $P_{\text{a,CO}_2}$ varies with metabolic rate and ventilation; the latter depending on the CO_2 chemoreflex sensitivity of the individual (Duffin, 2011). Such chemoreflex-mediated increases in ventilation, responding to the CO_2 in the inspired gas, moderate the rise in $P_{\text{a,CO}_2}$. Subjects with high chemoreflex sensitivities have greater increases in ventilation and, consequently, a lower $P_{\text{a,CO}_2}$ stimulus for CVR (Ainslie & Duffin, 2009). As a result, for inspired CO_2 fractions of 5% or less, the CO_2 -mediated ventilatory response may produce an unchanged, or even lowered $P_{\text{a,CO}_2}$ (Baddeley *et al.* 2000). Furthermore, any increased ventilation also increases arterial P_{O_2} ($P_{\text{a,O}_2}$) even if the inspired P_{O_2} remains constant (Floyd *et al.* 2003; Prisman *et al.* 2008; Mark *et al.* 2010). For example, Wise *et al.* (2007) found a 15 mmHg increase in P_{O_2} when subjects breathed 5% CO_2 in air. If BOLD MRI is used to measure CVR under these conditions, this rise in $P_{\text{a,O}_2}$ would result in a small but measurable $\sim 0.3\%$ increase in the BOLD signal (Prisman *et al.* 2008), which is about 10% of typical signal changes seen with a 10 mmHg increase in P_{CO_2} .

To circumvent this problem it has been argued that, regardless of the ventilatory response to administered CO_2 , end-tidal P_{CO_2} ($P_{\text{ET,CO}_2}$) is a suitable surrogate for $P_{\text{a,CO}_2}$, and therefore $P_{\text{ET,CO}_2}$ can be used as the stimulus for CBF (Hoskins, 1990; Ringelstein *et al.* 1992; Mark *et al.* 2011). In young healthy people, the gradient between $P_{\text{ET,CO}_2}$ and $P_{\text{a,CO}_2}$ may indeed be small – on average – but it is nevertheless unknown. The gradient between arterial and end-tidal measurements (Robbins *et al.* 1990) varies in an individual with age (Miller & Tenney, 1956; Badgwell *et al.* 1987), exercise (Jones *et al.* 1979), body position (Barr, 1963), and disease (Yamanaka & Sue, 1987; Liu *et al.* 1995; Prause *et al.* 1997). Even the assumption of a constant relation between $P_{\text{ET,CO}_2}$ and $P_{\text{a,CO}_2}$ may not be correct. In patients with lung disease, changes in $P_{\text{ET,CO}_2}$ and $P_{\text{a,CO}_2}$ are concordant only about 70% of the time (Russell & Graybeal, 1994; Grenier *et al.* 1999; McDonald *et al.* 2002). Thus, providing a fixed known inspired CO_2 fractional concentration and measuring the $P_{\text{ET,CO}_2}$ cannot standardize the $P_{\text{a,CO}_2}$ stimulus, and is therefore unsuitable for measuring CVR quantitatively.

Rebreathing. Rebreathing exhaled gas is a traditional means of increasing P_{CO_2} (Read, 1967). Its main advantages are that no external source of CO_2 is required and the basic apparatus, consisting of an exhaled gas reservoir and gas sensors are readily available. It lends itself to testing CVR as there is equilibration between arterial, and alveolar P_{CO_2} , which can be measured with each breath. The $P_{\text{ET,CO}_2}$, and hence the $P_{\text{a,CO}_2}$, can span a

range of values potentially enabling the exploration of the dynamic relationship between $P_{\text{a,CO}_2}$ and CBF.

However, there are a number of caveats which, if not addressed, limit its usefulness. First, during rebreathing, oxygen is continuously consumed from the reservoir changing the P_{O_2} , which should be kept constant for CVR measurement. Second, $P_{\text{a,CO}_2}$ values, and hence CBF are changing continuously. This ramp stimulus is not suitable for use with measures of CBF that drift over time such as BOLD MRI, or that require steady states like arterial spin labeling (ASL) (Hajjar *et al.* 2010); if CBF measures with high time resolution are used, they must be matched to the constantly changing $P_{\text{a,CO}_2}$. Third, the rate of rise of P_{CO_2} is difficult to predict or control; depending on the individual's rate of CO_2 production and the size of the rebreathing reservoir. Smaller reservoirs provide a more rapid rise in P_{CO_2} but may not provide adequate volume reserve for the larger tidal volumes as P_{CO_2} rises (Russo *et al.* 2000), and larger reservoirs prolong the rise time of P_{CO_2} , confounding measures of CBF that drift over time. Finally, contrary to the assertion that rebreathing quickly leads to a detectable 'steady state plateau' (Russo *et al.* 2000; Glodzik *et al.* 2011; Lee & Lee, 2011), complete rebreathing does not result in a plateau of P_{CO_2} as long the subject is alive (and producing CO_2). Plateaus are only reached with incomplete rebreathing, such as the addition of a dead space (Glodzik *et al.* 2011) or as a result of holes in the rebreathing bag (Lee & Lee, 2011). Moreover, such plateau P_{CO_2} values are not predictable (SD in one study was 3.5 mmHg; Lee & Lee, 2011), and the time constants for reaching actual plateaus in these cases are typically very long, and seldom attained (see Fig. 1 in Lee & Lee, 2011).

From these considerations it is apparent that rebreathing requires modification before it can be used to provide a stimulus for CVR measurement. Indeed, using rebreathing to measure the distribution of cerebral blood flow using BOLD MRI imaging is not appropriate; methods of maintaining a constant P_{O_2} , and compensating for its effect on BOLD signal have not been developed. However, a specialized rebreathing method where isoxia is maintained during rebreathing has been developed for measuring the characteristics of the respiratory chemoreflexes (Duffin, 2011) and is suitable for CVR measurements using TCD. Originally modified from the rebreathing method proposed by (Read, 1967) and first described by (Casey *et al.* 1987), the isoxic rebreathing method has been extensively reviewed (Jensen *et al.* 2010; Duffin, 2011). In this method, $P_{\text{ET,CO}_2}$ and $P_{\text{a,CO}_2}$ are equivalent, satisfying the requirements for a known measured hypercapnic stimulus, with end-tidal P_{O_2} ($P_{\text{ET,O}_2}$) maintained constant. Recently this method has been adopted for use with TCD to measure blood flow velocity in a number of studies (e.g. Fan *et al.* 2010; Skow *et al.* 2013) and to describe the sigmoidal response to CO_2 (Claassen *et al.* 2007; Battisti-Charbonney *et al.* 2011).

Dynamic end-tidal forcing. The first computerized method to control P_{ET,CO_2} and P_{ET,O_2} was developed by Swanson & Bellville (1975) and termed dynamic end-tidal forcing (DEF). It is implemented using a computer-controlled gas blender (Robbins *et al.* 1982*b*) with a feedback algorithm (Robbins *et al.* 1982*a*) to control the inspired concentration of source gases O_2 , CO_2 and N_2 , to target P_{ET,CO_2} and P_{ET,O_2} . To produce a sequence of changes in P_{ET,CO_2} and P_{ET,O_2} a predictive algorithm is used to calculate the inspired gas fractional concentrations necessary to produce the target end-tidal tensions. DEF has been used to control P_{ET,CO_2} and P_{ET,O_2} during BOLD MRI imaging for CVR in a single study of four subjects (Wise *et al.* 2007) and of coronary vascular reactivity in 15 subjects (Beaudin *et al.* 2011).

In operation, the difference between the target and actual end-tidal tensions is assessed on a breath-by-breath basis, and using the known inspired concentrations of gases, the errors in the inspired concentrations can be determined. With that error information and a prediction of the size of the next breath, the system controller calculates the corrections to the programmed inspired gas concentrations necessary to attain the target end-tidal tensions in the next breath. These corrective actions are made continuously when steady target end-tidal tensions are required. However, for rapid changes, the controller shortens the time for transition by overcompensating, and then reverts to maintaining corrective actions. For rapid decreases in P_{ET,CO_2} the method depends on increased ventilatory drive or voluntary hyperventilation.

While the DEF system is capable of controlling end-tidal gasses, we suggest that it is not suitable for clinical CVR testing for a number of reasons. First, implementation of the DEF system poses some difficulties. It is resource intensive, requiring an extensive and complex infrastructure (see Fig. 1 of Wise *et al.* 2007) because the system requires high flows ($\sim 70 \text{ l min}^{-1}$) in order to exceed the subject's peak inspiratory flow, which must be humidified before delivery to an open cone from which the subject breathes. The installation must therefore include the means to store and supply large volumes of humidified gas at high flows via large diameter tubing, and a breathing circuit with MRI-compatible electronic components such as flow sensors. Second, its performance in terms of accuracy and speed need improvement. Step increases in P_{ET,CO_2} are slow; despite utilizing 'overpressure' the time to transition to a new P_{ET,CO_2} can take more than a minute (Beaudin *et al.* 2011). And for maintaining steady target tensions, the DEF control system prediction-correction algorithm has inherent breath-to-breath variations in P_{ET,CO_2} and P_{ET,O_2} of up to 2 mmHg (see Fig. 2, panel 2, in Wise *et al.* (2007) and Fig. 1 in Beaudin *et al.* (2011)). Third, as previously discussed, the relation between P_{ET,CO_2} (the measured value) and P_{a,CO_2} (the actual CVR stimulus) is not known in an individual (e.g. St Croix *et al.*

1995). Finally, although the DEF system also incorporates safety features consisting of monitors for heart rate, pulse oximetry, inspired O_2 concentration, and the presence of a back-up O_2 source, maintaining safety is challenging because a failure of the O_2 flow provides a totally anoxic inspiratory gas flow that must be detected and rectified immediately to prevent harm.

Prospective end-tidal targeting. Approximately 15 years ago, our laboratory set out to develop an automated device that would reliably administer a repeatable change in P_{a,CO_2} , while maintaining isoxia, with minimal subject cooperation. As BOLD MRI signal was the intended surrogate for CBF, the equipment needed to be MRI compatible and compact. It also had to be economical, and inherently safe. The method that emerged became known as prospective targeting of P_{ET,CO_2} and P_{ET,O_2} (Slessarev *et al.* 2007; Blockley *et al.* 2011; Alderliesten *et al.* 2013). The core feature of the method is the capability of controlling the amount and content of gas entering the lung gas exchange region independent of the tidal volume and pattern of breathing. This capability was achieved by using a simple 3-valve manifold and circuit that, with control of the flow of gas to the circuit, produced a sequential delivery of inspired and previously exhaled gas. Its operation is illustrated in Fig. 2.

As Fig. 2 shows, gases from the inspiratory reservoir and from the expiratory reservoir are delivered sequentially

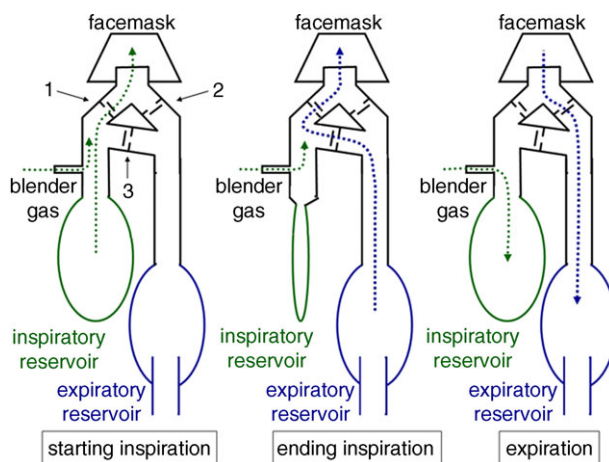


Figure 2. The sequential gas delivery circuit (SGD) operation
The valve manifold has an inspiratory valve (1), an expiratory valve (2) and a cross-over valve (3). The latter has a slightly greater opening pressure than the other valves and, when open allows gas to cross from the expiratory to the inspiratory limb. During expiration, gas delivered by the gas blender enters the inspiratory gas reservoir (green dotted line), and exhaled gas enters the expired gas reservoir (blue dashed line). During inspiration, gas is drawn from the stored blender gas in the inspiratory gas reservoir (green dotted lines). If the inspired volume exceeds the volume in the inspiratory reservoir, the balance comes from the expiratory gas reservoir via the cross-over valve (blue dashed line).

during inspiration (Sommer *et al.* 1998; Somogyi *et al.* 2005; Slessarev *et al.* 2007). The functional significance of this arrangement is that the rebreathed gas in the expiratory reservoir, having already equilibrated with the blood, provides no gradient for any further gas exchange, i.e. rebreathed gas is neutral with respect to pulmonary gas exchange. This sequential gas delivery (SGD) means that the gas supplied to the inspiratory reservoir is the only source of gas available for gas exchange in the lung. Provided the subject empties the inspiratory reservoir for each breath, the output of the gas blender is the alveolar ventilation, regardless of actual breath size or breathing pattern.

This prospective targeting methodology confers four major advantages. First, with alveolar ventilation precisely administered by the gas blender, gas tensions can be accurately targeted because P_{ET,CO_2} and P_{ET,O_2} are determined by the gas fractional concentrations in the alveolar ventilation (Slessarev *et al.* 2007). Moreover, the targeting of each gas is independent of the other as well as independent of both the minute ventilation and the breathing pattern of the subject, as Fig. 3 illustrates. Second, gas blender flows to the SGD circuit approximate resting ventilation, so that more compact

gas sources can be used, and gas utilization is minimized and expenses reduced. Also the system operation is quiet, and gas humidification can easily be achieved. Third, the prospective nature of the system targeting eliminates the need for a feedback correction mechanism thereby markedly reducing the computational complexity while minimizing the breath-to-breath variability of P_{ET,CO_2} . Fourth, a minimal degree of cooperation is required of the subjects; all they need to do is breathe so that their ventilation exceeds the blender gas flow to the inspiratory reservoir. Finally, and most important, because the SGD circuit includes an element of expired gas rebreathing, alveolar inhomogeneities are reduced and, as a result, the gradient between P_{ET,CO_2} and P_{a,CO_2} is reduced to the range of instrument measuring error (0.5 ± 1.7 mmHg; $P = 0.53$; Ito *et al.* 2008; Fierstra *et al.* 2011) under steady conditions.

Until now it has not been possible to accurately specify the P_{a,CO_2} stimulus, and so it was not known how precisely the BOLD MRI signal follows the change in P_{a,CO_2} . We found that after synchronizing the phases of the P_{ET,CO_2} and BOLD signals, the waveforms track precisely, voxel-by-voxel as shown in Fig. 4. This observation demonstrates that any inaccuracies of the

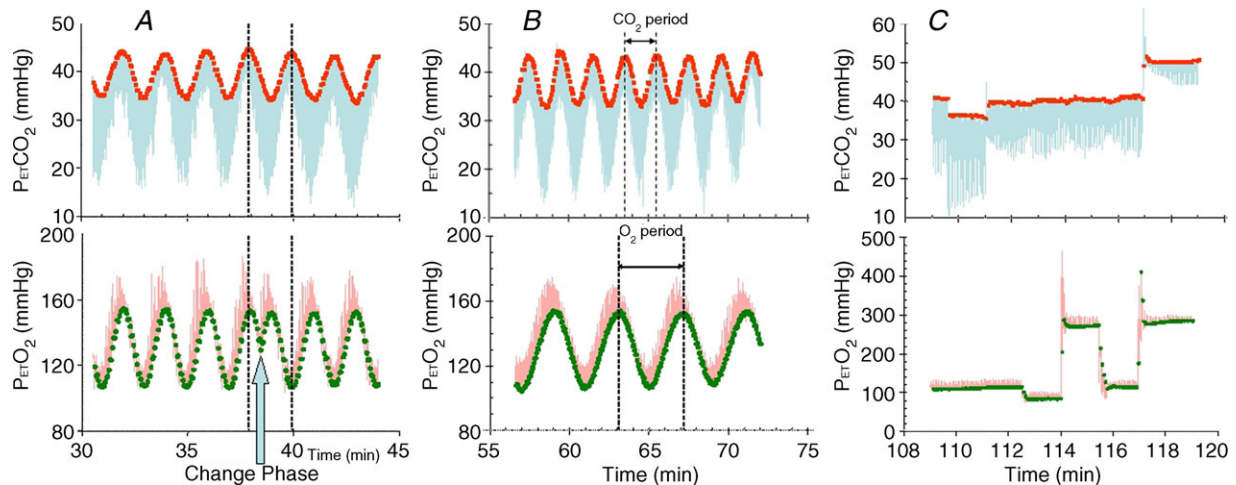


Figure 3. Examples of P_{ET,CO_2} and P_{ET,O_2} control in a subject using an SGD breathing circuit and prospective end-tidal targeting

The continuous traces from sampling at the mask are P_{CO_2} (blue upper) and P_{O_2} (red lower). End-tidal values are P_{ET,CO_2} (red filled squares) and P_{ET,O_2} (green filled circles), each representing a single breath. A, sinusoidal changes of P_{ET,CO_2} and P_{ET,O_2} are implemented in phase until the blue arrow, when the phase of the P_{ET,O_2} is changed 180 deg. B, sinusoidal changes of P_{ET,CO_2} and P_{ET,O_2} are implemented with the period of P_{ET,O_2} twice that of P_{ET,CO_2} . C, simultaneous square wave changes in P_{ET,CO_2} and P_{ET,O_2} are implemented independently of each other. In the sinusoidal patterns, the target P_{ET,CO_2} and P_{ET,O_2} change with each breath. The algorithm used to reach these targets is context sensitive, that is, it takes into account the current gas concentrations in the lung as well as the target history. This means that the set of flows and inspired gas concentrations differ – even for the same recurrent end-tidal target values, whether they be in a sinusoidal sequence or steady target level. The algorithm uses the baseline P_{CO_2} , and resting CO_2 production and O_2 consumption to calculate inspired gas parameters. Baseline P_{CO_2} is based on the P_{ET,CO_2} during rest. Resting CO_2 production and O_2 consumption are calculated from a nomogram based on sex, height and weight. Errors in presumption of CO_2 production or O_2 consumption, or changes in these due to changes in activity or muscle tone, result in target values drifting over time, as can be seen in A and C.

independent variable P_{a,CO_2} will result in inaccuracies of the CVR measurements, and so impede interpretation. The computer controlled gas blender and SGD circuit can be used to prospectively target P_{a,CO_2} , which is accurately and precisely known at breath-by-breath intervals through the measurement of P_{ET,CO_2} .

However, as well as the advantages described above, the prospective targeting system, has a number of limitations associated with its application. As discussed in the caption in Fig. 3, initialization of the targeting software requires unmeasured parameters such as baseline P_{a,CO_2} , CO_2 production and O_2 consumption; incorrect assumptions are a source of error in targeting. In addition, the achievement of precise targeting has fastidious technical requirements; the most important of which is that the interface with the subject be absolutely airtight. Skin tape such as Tegaderm (3M, St Paul MN, USA) is used around the mask by most investigators. Although the breathing circuit is simple in design, it nevertheless has strict performance requirements, particularly in the competence of the valves and the condition of the reservoirs during targeting (e.g. collapse of the inspiratory reservoir on inspiration; venting the expiratory reservoir without contaminating its contents with air). The system requires preparation of two custom blend gases containing different concentrations of O_2 , CO_2 and N_2 to circumvent the possibility of administering hypoxic gas. Subjects must cooperate to the extent that they empty the inspiratory reservoir each breath so that, on occasion, they must either increase their minute ventilation on command; alternatively, the user can reduce the blend gas flow (whereby the gas concentrations automatically adjust to maintain targeting). Thus, the

gas blender and associated software, like that of DEF system, are too complex for most laboratories to assemble and achieve sophistication and safety. Neither are they currently regulatory approved commercially available devices. Nevertheless, we have made every effort to selectively make available hand-assembled devices for collaborative research, resulting in about 60 publications in the peer reviewed literature enabled by the device.

Conclusions

Measures of CBF are now highly reproducible, but variability of the vasoactive stimulus remains a major impediment in the pursuit of a standardized cerebrovascular test employing CVR measurement. Reducing this source of variability will further advance the art towards the goal of generating useful comparisons of CVR maps between subjects or groups of subjects, as well as the ability to follow CVR changes in a given subject over time. After reviewing the various vasoactive stimuli, we conclude that while the modified rebreathing method may be used to measure CVR with TCD, only the computer controlled gas delivery DEF and prospective end-tidal gas targeting systems are capable of providing reliable and repeatable vasoactive CO_2 stimuli for both TCD and clinically available imaging techniques such as BOLD MRI that produce CVR maps. These latter methods, therefore, may be candidates for incorporation into standardized CVR measurements; of these systems, prospective end-tidal targeting is the most practical for routine testing (Spano *et al.* 2012) with all measurement modalities.

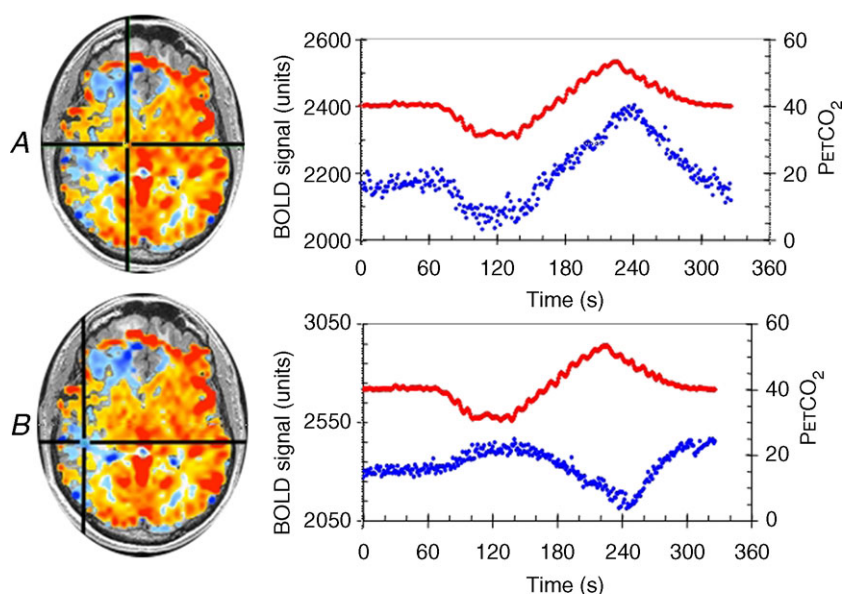


Figure 4. Voxel tracking of P_{ET,CO_2}

CVR maps, constructed as for Fig. 1, are shown on the left with chosen voxels indicated by the cross-hairs. The right side shows graphs of the time course of the chosen voxels BOLD signals (blue dots) in response to the changes in P_{ET,CO_2} (red dots). A, a voxel with positive CVR. B, a voxel with negative CVR (vascular steal). In each case the BOLD signals track the P_{ET,CO_2} stimulus, indicating that a precise and accurate measurement of CVR requires accuracy and precision of the P_{a,CO_2} stimulus as well as the surrogate measure of cerebral blood flow.

Appendix

A selective primer in respiratory physiology

Terminology	
Alveolar ventilation	The gas in the lungs contributing to gas exchange with the blood
Breath hold index	P_{CO_2} increase divided by breath-hold time
CO_2	Carbon dioxide
F_{CO_2}	Fractional concentration of CO_2 , the proportion of CO_2 in a gas
Hypercapnia	Higher than normal P_{CO_2}
Hypocapnia	Lower than normal P_{CO_2}
Minute ventilation	Gas volume exchanged in the lungs in litres per minute
P_{CO_2}	Partial pressure of CO_2
Peak inspiratory flow	Peak flow of gas during a breath
$P_{\text{ET},\text{CO}_2}$	End-tidal partial pressure of carbon dioxide
Rebreathing	Inhaling previously exhaled gas
Ventilatory drive	The urge to breathe

Carbon dioxide (CO_2) is produced in the body by metabolism, so that CO_2 levels in the blood are determined by the balance between the rate of production by the tissues and the rate of elimination through the lungs.

The partial pressure of CO_2 (P_{CO_2}), in a gas mixture is F_{CO_2} times the ambient pressure (Henry's law). Partial pressure determines the movement of gases in the body, so the concentration of CO_2 in the blood is a function of P_{CO_2} (CO_2 dissociation curve). The partial pressure of CO_2 in arterial blood is represented as P_{a,CO_2} , which is considered to be the independent variable affecting the flow resistance of cerebral vessels.

End-tidal P_{CO_2} ($P_{\text{ET},\text{CO}_2}$) is the partial pressure of CO_2 in the gas at the end of an exhalation. It is that which has fully equilibrated with the blood and so is of interest.

Breath-holding (holding one's breath) reduces the rate of elimination of CO_2 to zero. As a result CO_2 accumulates in the blood and its fractional concentration and partial pressure rises.

Rebreathing is the inhalation of previously exhaled gas. As this gas has already equilibrated with the blood, it does not participate in gas exchange. Therefore the rebreathed portion of the breath does not contribute to alveolar ventilation. If the entire breath consists of previously exhaled gas, the result is the same as breath-holding; there is a steady accumulation of CO_2 in the blood. The rate of rise of CO_2 in the blood depends on the body's CO_2 production and the body size. There is also a steady consumption of oxygen (O_2), and without O_2 replacement, the O_2 concentration in the blood falls continuously. Rebreathing methods therefore require the addition of O_2 to maintain blood O_2 levels.

Inhaling CO_2 also adds to the accumulation of CO_2 in the blood. When the inspired gas has a constant concentration of CO_2 , the CO_2 level in the blood is a function of both this inspired concentration (which tends to raise the concentration of CO_2) and the level of ventilation (which tends to lower it). Since the level of ventilation to a given inspired concentration of CO_2 varies between people, the blood concentration of CO_2 to a given inspired concentration will vary.

Chemoreflexes control the partial pressure of CO_2 in the blood. If the P_{CO_2} exceeds a threshold the central and peripheral chemoreflexes effect a reflex increase in ventilation to restore resting P_{CO_2} .

The relationship between $P_{\text{ET},\text{CO}_2}$ and P_{a,O_2} is not constant. In some healthy people they may be closely related, but the relationship is not reliable. The only exception is when CO_2 is inhaled at the end of a breath. Our system of prospective targeting of $P_{\text{ET},\text{CO}_2}$ includes some rebreathing at the end of inhalation, which has been shown to equalize the $P_{\text{ET},\text{CO}_2}$ and P_{a,CO_2} . In this case, the $P_{\text{ET},\text{CO}_2}$ is an accurate surrogate for the P_{a,CO_2} , the independent variable affecting cerebral blood flow.

Tidal volume is the volume of a breath. To generate a breath the diaphragm and chest muscles contract. The flow of gas into the lungs starts from 0, reaches a maximum and ends at 0 flow just before exhalation. The maximum flow at rest in adults can be 50 l min^{-1} . It can be 2 to 3 times this with greater breathing efforts. When the subject breathes gas provided by a mask that is open to atmosphere (such as a regular oxygen mask), and the flow of gas does not exceed this peak inspiratory flow, gas from the room is drawn into the mask and dilutes the administered gas. Typical maximum oxygen flows into the mask are $5\text{--}15 \text{ l min}^{-1}$. Maximum flow that can be conducted in standard oxygen tubing is about 25 l min^{-1} . Meeting peak inspiratory flow rates requires gas flows of 70 l min^{-1} or more.

Controlling lung gas concentrations is difficult. After an exhalation there is a large volume of gas left in the lung called the 'functional residual capacity', or FRC. The FRC is typically very large ($\sim 3 \text{ l}$ in an adult) compared to the breath size ($\sim 0.5 \text{ l}$). As a result it takes many breaths to 'wash out' the current gas concentration in the FRC and replace it with gas of another concentration. Computerized gas delivery systems have developed strategies to optimize this exchange.

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Additional information

Competing interests

J.A.F. is Chief Scientist and J.D. is Senior Scientist at Thornhill Research Inc. (TRI), a spin-off company from the University Health Network that developed the RespirActTM. RespirActTM is currently a non-commercial research tool made available for this research by TRI.